April 28, 2000

MEMORANDUM

SUBJECT: **MALATHION:** Response to Memorandum (Malathion: Evaluation of Hazard Identification Assessment Review Committee Reports Regarding Scientific Issues Presented by Dr. Dementi, March 28, 2000; V. Dobozy to M. Stasikowski, J. Rowland and E. Doyle)

FROM: K. Clark Swentzel, Chief
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TO: Margaret Stasikowski, Director
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and

Jess Rowland, Co-Chair
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division

I am responding to the subject memorandum from Dr. Dobozy, as requested by the Health Effects Division (HED) senior management, to address the suggestions and questions that were included as a result of her evaluation of relevant Hazard Identification Assessment Review Committee (HIARC) reports. The objective of this response is to elucidate these reports by answering questions as well as providing additional rationale and information that Dr. Dobozy considered appropriate. Dr. Dobozy focused primarily on the HIARC document, dated December, 22,1998, which described the HIARC's evaluation of responses from an External Peer Review Panel of

Toxicologists to questions submitted to them by Dr. Brian Dementi. The responses in this memorandum will be applicable to the December 22, 1998 memorandum unless otherwise noted and can be attached to it as a supplement.

The following issues were addressed by the External Peer Review Panel. Their responses to these issues, which were discussed by the HIARC during meetings on August 18, 20 and 27, 1998, did not convince the HIARC to change any of their relevant decisions with respect to non-cancer risk assessments.

I. Issues addressed by the External Peer Review Panel of Toxicologists

A. Food Quality Protection Act (FQPA) 10X Safety Factor for Protection of Infants and Children

Dr. Dobozy: Clarification is needed for the body weight decreases observed in the 2-generation reproduction study in rats.

Response: In the F0 generation, after the first mating, body weight in females was decreased below controls at 7500 ppm (703 mg/kg/day) on gestation days 7, 14, 20 and during the entire lactation period and at 5000 ppm on lactation day 14. After the second mating, body weight was decreased at 7500 ppm on lactation days 0 and 21. In the F1 generation, body weight was decreased below controls at 7500 ppm(612 mg/kg/day) during the entire treatment period for males and during premating weeks 1-3, 5 and 7-11 for females.

Dr. Dobozy: Indicate dosages in the subchronic and chronic toxicity studies in rats at which cholinesterase inhibition (ChEI) was observed as compared to the body weight decrements observed in the 2-generation reproduction study.

Response: The treatment level at which parental body weight decrements were observed was substantially (more than 10 fold) greater than the treatment levels at which plasma cholinesterase inhibition was seen in the chronic rat study with Malathion (29 and 35 mg/kg/day in males and females, respectively) and nearly twice the treatment levels (359 and 415 mg/kg/day in males and females, respectively) at which RBC cholinesterase inhibition was observed in that study. Plasma and RBC cholinesterase inhibition was seen in a subchronic study (13 weeks of dietary administration) with Malathion in rats at dosages of 352 and 395 mg/kg/day in males and females, respectively.

Dr. Dobozy: Give the basis for the statement in the August 6, 1998 FQPA report that estimation of the test substance intake in pre-weaning animals is likely to more than double the adult intake.

Response: During the later period of lactation (the period in question), young rats consume approximately twice the diet per unit body weight as an adult rat consumes. Also, it is assumed that Malathion was consumed concomitantly in the milk by rats in late lactation, as has been assumed for other compounds in this class (organophosphates).

During the discussion of this issue during the August 18, 20 and 27, 1998 meetings, it was noted that data from ruminant metabolism studies submitted to the Agency indicated that Malathion was not detected in the milk of these animals, however, there are data from reports in the open literature that show detectible levels in the milk of cows exposed to Malathion (e.g., Claborn et al.[1956]. J. Agr. Food Chem.4(11):941); Milhaud et al. [1971]. Rec. Med. Vet. 147 (10): 1053-1061).

Dr. Dobozy: Referred to Attachments 17 and 18 in which Dr. Dementi refers to studies from the open literature which indicate increased sensitivity of young versus adult animals. If they were discussed, they should be included in the HIARC report.

Response: These references were not presented to the HIARC for review, but were cited in a Minority Report submitted to the HIARC after this issue had been discussed. Additional discussion of this issue follows in Section III (Other Issues)

Dr. Dobozy: Discuss the completeness of the data base relative to the FQPA Safety Factor recommendation. State the HIARC's conclusions about the External Peer Review Panel's responses and how they impacted the FQPA Safety Factor determination.

Response: All of the relevant studies on Malathion were considered before the HIARC made their recommendation regarding the FQPA Safety Factor. The HIARC considered the toxicology data base to be complete with respect to those core studies that are necessary for FQPA assessment. These studies are acute and subchronic neurotoxicity studies in rats, pre-natal developmental toxicity studies in rats and rabbits and a 2-generation reproduction study in rats.

The External Peer Review Panel members presented their knowledge and opinions in the areas of concern and expressed their personal biases regarding the interpretation of data and the application of an FQPA Safety Factor. The HIARC did not believe that any changes to their recommendation, based on the opinions and/or information provided by the panel, were warranted. This conclusion was reached after extensive discussions in the HIARC in meetings on August 18, 20 and 27 which focused on the External Peer Review Panel's responses/comments with respect to their potential impact on the FQPA assessment as well as the overall risk assessment.

B. Hazard Identification/Acute Oral (One-Day)

Dr. Dobozy: The HIARC reports should be clear as to why the acute neurotoxicity (general population) and the developmental studies (general and/or females 13-50) were not useful for the acute dietary risk assessment.

Response: The HIARC did not find a suitable endpoint for risk assessment that could be attributed to a single exposure in either developmental study (rat or rabbit) or the acute neurotoxicity study. The HIARC explains why effects observed in the rabbit development study were not appropriate for acute dietary risk assessment.These are the studies that are typically examined using the weight-of-evidence approach recommended by the HIARC Standard Operating Procedure as specified in the *Toxicology Enpoint Selection Document, August, 1998.*

The December 17, 1997 HIARC report explained that the acute neurotoxicity study in rats is not appropriate because of low confidence in the assessment of cholinesterase activity. The lack of dose response and a clear NOAEL for this biomarker constituted an inherent weakness of this

study. The Data Evaluation Record indicates that there is a poor dose response relationship for plasma and RBC inhibition for both sexes. In plasma, cholinesterase was inhibited at the low- and high-doses but not the mid-dose on day 7 in males; in females the greatest inhibition occurred on day 7 in the low-dose group. The RBC cholinesterase data show that inhibition occurred at the high-dose at day 7 in both sexes. Dr. Dobozy stated that this study was rejected in favor of a repeated dose study for acute dietary risk assessment, however, the HIARC actually used a weight-of evidence approach from the Malathion data base to select a NOAEL that would be protective against a single exposure.

Dr. Dobozy: Why is an acute dietary risk assessment necessary?

Response: It is generally recognized that this class of compounds (organophosphates) can inhibit cholinesterase after a single exposure. One could argue that an acute dietary risk assessment may not be required for malathion since the principal toxicological effect of concern (cholinesterase inhibition) was seen only at very high doses (500, 1000 or 2000 mg/kg) after a single dose in the acute neurotoxicity study, no adverse maternal or developmental effects were seen in the prenatal developmental toxicity studies in rats and rabbits and an endpoint attributable to a single dose was not evident in the other toxicity studies. However, the HIARC chose to be conservative and selected a no-effect dose for acute dietary risk assessments for the protection of the general population, including infants and children, from any potential acute exposure to Malathion.

Dr. Dobozy: Why isn't the LOEL (based on increased resorption sites) in the developmental toxicity study in rabbits acceptable for acute dietary risk assessment?

Response: The HIARC report (December 7, 1997) states that the increase in resorption sites/dam at 50 mg/kg/day was not considered to be an appropriate endpoint because the incidence was only slightly increased and was considered by the Committee to be of no meaningful toxicological significance with respect to acute dietary risk assessment. In addition to the statements in the HIARC report, it should be noted that these data did not fit a clear dose response and the increase seen at 50 mg/kg/day was not statistically significant. Although the NOAEL/LOAEL for both fetal and maternal effects were the same, 25 and 50 mg/kg/day, respectively, the HIARC did not use the 25 mg/kg/day dose as an endpoint for acute dietary for females 13-50 because the evidence for direct fetal effects was weak. Specifically, the increase in percent resorptions from 50 to 100 mg/kg/day was not dose-related, the number of live fetuses per group was not significantly affected at any dose (they were increased at the lowest dose of 25 mg/kg/day, decreased at 50 mg/kg/day and similar to controls at 100 mg/kg/day) and there was no apparent effect on any other fetal parameter. The HIARC judged the increase in resorption sites to be a marginal effect at best, and therefore chose not to use this endpoint for risk assessment.

It is not unusual for the HIARC to reject certain endpoints for risk assessment, especially if they appear to involve threshold effects or effects that are not considered toxicologically significant with respect to risk assessment.

Dr. Dobozy: Give the rationale for selecting 50 mg/kg as the NOEL for the risk assessment.

Response: The HIARC report explained that the data base was examined and no toxicologically or statistically significant effect was found at a dosage of 50 mg/kg that could be attributed to a single exposure. It was the Committee's opinion that this dose is both conservative and protective.

Dr. Dobozy: What maternal endpoint was used for the risk assessment?

Response: The Summary of Toxicology Endpoint Selection table is somewhat misleading since a weight-of-evidence approach was used and several studies were considered before a NOEL was selected. The maternal toxicity seen in the rabbit developmental study was decreased body weight gain, however, for there was no appropriate endpoint for a single exposure. As indicated before, a weight-of-evidence approach was used which involved consideration of both developmental toxicity studies as well as the acute neurotoxicity toxicity study. Clarification of this table will be addressed by the Agency.

Dr. Dobozy: The record should reflect if individual maternal body weight data from the rabbit developmental toxicity study have been have been reviewed and evaluated by the Agency.

Response: It is evident in the HIARC report that the External Panel looked at these data and did not consider them relevant for a single exposure at 50 mg/kg. The toxicology reviewer should indicate whether or not the data have been reviewed by the Agency. It was assumed that the reviewer considered these data when the Data Evaluation Record was prepared.

C. Hazard Identification/Chronic Dietary (RfD)

Dr. Dobozy: Was the human study re-evaluated in light of the peer review panel's responses?

Response: The HIARC evaluated the human study and did not find it to be acceptable based on several deficiencies which are listed in their report. The panel was split on the acceptability of this study for risk assessment. This is a moot point since current Agency policy does not permit the use of human data for risk assessments.

Dr. Dobozy: Explain why a 3-month study to define the NOEL for ChEI in female rats is not needed.

Response: This study is not needed because a NOEL of 50 ppm for ChEI has been established in both sexes in a subchronic neurotoxicity study in rats as well as a chronic toxicity study in rats following a 6-month dietary level adjustment from 100 to 50 ppm. The NOELs for both of these studies are given in the HIARC report.

D. **Subchronic Inhalation Study**

Dr. Dobozy: The HIARC report is unclear as to the Committee's final decision about the inhalation endpoint (any time period) and the basis for it.

Response: The December 17, 1997 HIARC report indicated that the endpoint was based on the lowest dose tested (0.1 mg/L) in a subchronic inhalation toxicity study. This dose is considered a LOAEL based on inhibition of plasma and RBC cholinesterase inhibition in females and histopathological lesions of the nasal cavity and larynx in both sexes. This study was used for all time periods because it is the only inhalation toxicity study available to the HIARC at that time. The review of a two-week range-finding study, which was subsequently provided to the HIARC and discussed at a later meeting, did not establish NOAELs for cholinesterase inhibition or histopathology findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L.

Dr. Dobozy: The HIARC concluded that a new inhalation study is required , however, it is unclear as to its duration and method of administration, i.e. whole body or nose-only.

Response: The HIARC requested a subchronic (90-days) inhalation study in rats with nose only exposure.

Dr. Dobozy: The last sentence under Question 5 is unclear. The question did not ask if a 10X should be applied to the study, as indicated in the sentence.

Response: Question 5 (submitted to the External Panel): *Other than contributing to the completeness of the Malathion data base, does this study provide any support for discounting a 10X Safety Factor imposed under FQPA for the protection of infants and children?*

The purpose of that sentence was to emphasize that decisions made with respect to the FQPA safety factor are not typically based on one toxicology study since dietary and residential exposure must also be considered. The HIARC does not consider this study appropriate for FQPA assessment because the study was conducted in adult animals which were not pregnant, therefore, it is not possible to use this study to determine possible susceptibility in young animals.

The HIARC concluded that discussion about the FQPA Safety Factor is neither applicable nor appropriate for this study (i.e. the question should never have been asked).

E. Acute Neurotoxicity Study (Retinal Histopathology)

Dr. Dobozy: The rationale for not requesting that retinal histopathology data for rats in the intermediate dose group is the same in both the December 17, 1997 and December 22, 1998 HIARC reports.

Response: The HIARC considered the rationale provided in a December 3, 1997 report by the *ad hoc* Neurotoxicity Subgroup to be appropriate. The External Panel's responses to the question with respect to a request for this data were considered during the August 18, 20 and 27 meetings but did not convince the HIARC to change their conclusion.

Dr. Dobozy: The document should indicate what the Agency's position is on these lesions. The 1997 HIARC report (III. FQPA Considerations, 1. Neurotoxicity Data) refers to them as a equivocal neuropathological finding. The December 22, 1998 HIARC report should note if the August 1998 discussions altered this conclusion. It is noted by this evaluator that other lesions were reported in this study and designated as equivocal neuropathological findings. The high-dose male animal also had axonal degeneration in the lumbar root. Digestion chambers in the lumbar dorsal root fibers on one male and in the sciatic nerve and tibial nerve in another male were observed. These lesions were also designated as equivocal findings.

Response: The HIARC discussion of August 1998 did not result in a change of their conclusion about the retinal rosette observed in one male rat in the high-dose group. As noted above, the HIARC concurred with the conclusions in the December 3, 1997 report of the *ad hoc* Neurotoxicity Subgroup on this issue. The HIARC's description of the other noted lesions as equivocal neuropathological findings was based on the information in the Data Evaluation Record for this study. A different description would not be appropriate without reviewing the original study report to see how findings overlapped in individual animals.

Dr. Dobozy: Was Dr. Dementi's January 15, 1998 attachment (#7) considered by the HIARC?

Response: Dr. Dementi's attachment cited a study in the open literature: Mendoza, C.E. (1976) Toxicity and Effects of Malathion on Esterases of Suckling Albino Rats. *Toxicology and Applied Pharmacology* 35:229-238, that indicates increased sensitivity of young versus adult animals. This report was not reviewed by the HIARC since it was cited in a minority report which was submitted to the HIARC after this issue had been discussed. This report is discussed under Section III of this memorandum.

F. Subchronic Neurotoxicity Study (Recommendation for Additional Behavioral Effects Testing)

Dr. Dobozy: Since the NOEL in this study is higher than the LOEL in a non-guideline subchronic neurotoxicity study in female rats (reported by Desi et al., 1976), the Desi study should be reviewed and evaluated by the HIARC for its usefulness in risk assessment.

Response: The *ad hoc* Neurotoxicity Subgroup discussed the general subject of learning/behavior studies and also considered specific information pertinent to the Desi et al. Study in a November 3, 1997 meeting. In addition, a memorandum from R.C. Macphail (Chief, Neurobehavorial Toxicology Branch/HERL/EPA) to John Doherty (HED) and Brian Dementi (HED), dated May 4, 1995, was available which commented on the Desi et al. study and on the potential regulatory usefulness of further neurotoxicity testing of Malathion. The author of this report concluded that dietary Malathion reduced the speed and increased the number of errors of rats in a T-maze. MacPhail indicated that "whether this is a real effect of Malathion on learning is debatable. The effect is small (all rats really made errors with continued training) and not dose-related". The consensus of the *ad hoc* Neurotoxicity Subgroup was that, based on the presently available information, EPA should not ask for additional neurotoxicity studies on Malathion at this time, however, the option to request them in the future should remain open. The *ad hoc* Neurotoxicity Subgroup recommended that a literature search should be performed on 1) learning/behavior effects of organophosphates in general and 2) available information on Malathion in particular. The HIARC has not received any indication that this was ever done.

As Dr. Dobozy indicated, this issue is essentially moot since a developmental neurotoxicity study is required for Malathion under a Data Call-In dated September 10, 1999.

G. Cholinesterase Inhibition - Enhanced Sensitivity of Females

Dr. Dobozy: The HIARC report is not clear as to whether the entire data base was examined to address the question of possible gender sensitivity.

Response: The entire data base was examined by the *ad hoc* Neurotoxicity Subgroup when they addressed this issue. It was the conclusion of this group that the data base did not consistently show that females were more sensitive to Malathion than males.

Dr. Dobozy: It is unclear if any of the HIARC's conclusions about the questions for this issue are based on the *ad hoc* neurotoxicity subgroup's conclusions.

Response: The HIARC discussed this issue at the August 18, 20 and 27 meetings and concurred with the conclusions of the *ad hoc* Neurotoxicity Subgroup with respect to the questions on this issue.

Dr. Dobozy: Why was NOEL and NOAEL used in the same paragraph when discussing the cholinesterase findings?

Response: This is an oversight in the report which was written during the period when HED was in the process of changing the abreviations to describe a dosage level at which no adverse effects are observed.

II. Interpretation of the External Peer Review Panel's Responses to the Submitted Questions

Dr. Dobozy provided her interpretation of the responses from the External Peer Review Panel to questions which were submitted by the Agency and compared them to the HIARC's interpretations which are included in the December 22, 1998 report. There was substantial differences between Dr. Dobozy and the HIARC report on 2 of those responses.

1) Question submitted to the panel: Can the data from the reproduction study be considered adequate to address whether young or mature animals are more sensitive to Malathion, given the absence of ChE and behavioral assessments in adult and young animals?

HIARC report: The panel appears to agree in saying no to this question, i.e., data in the 2-generation reproduction study are not adequate to address the question of relative sensitivity of younger versus mature animals.

Dr. Dobozy: Drs. Decker and Dourson responded no. Dr. Hartung did not answer the question concerning the malathion reproduction study but responded generally. Therefore, it cannot be concluded that he answered no to this question.

2) Question submitted to the panel: Do the rabbit developmental toxicity and range-finding toxicity studies support a conclusion that a single oral dose of malthion as high as 50 mg/kg be without toxicological consequence in either maternal or developing organisms?

HIARC report: The panel did not think the Agency's acute dietary endpoint of 50 mg/kg was justified based on the rabbit data and thought that an acute oral study measuring cholinesterase would be better.

Dr. Dobozy: Dr. Dourson said the available data do not allow this question to be answered. Dr. Hartung said the available information is inconclusive at least for the developmental effect. Therefore, two of the three panel members did not answer the question directly. Although Dr. Decker answered the question no, his reference to the basis for his conclusion was not correct.

Response: It is my opinion that Dr. Dobozy's interpretations of the panel's responses to these 2 questions are more representative and clearly show the lack of consensus among the panel members in their answers to both questions.

III. Other Issues

A. Age-Related Susceptibility

There are currently on-going discussions with the registrant, Cheminova, regarding the design and conduct of the developmental neurotoxicity study in rats with malathion. As part of the additonal work that needs to be performed (as per the 9/10/99 Data-Call-In that initiated this effort), the registrant has agreed to assess comparitive cholinesterase data in adults and pups, following acute oral (gavage) administration of malathion. The results of this study, which will be conducted under GLPs, should serve to further the assessment of age-related susceptibility. Additionally, the results of the developmental neurotoxicity study will provide an assessment of potential quantitative and/or qualitative susceptibilitiy following pre- and/or postnatal exposure.

Dr. Dobozy referred to Attachments 17 and 18 from Dr. Dementi which cited open literature reports relevant to this issue. These attachments were submitted after the HIARC had discussed this issue. The following report was cited in Attachment 17: Mendoza, C.E. (1976) Toxicity and Effects of Malathion on Esterases of Suckling Albino Rats. *Toxicology and Applied Pharmacology 35:229-238.* The author used neonate LD50 data to demonstrate that 1-day old pups were 9 times more susceptible than 17-day old pups and 5 and 4 times more susceptible than 12- and 6- day-old pups, respectively. While these data are noteworthy, they do not in and of themselves jutify the retention of an FQPA safety factor on the basis of age-related susceptibility or sensitivity. The susceptibility was observed at lethal doses (the endpoint of the LD50 studies is death). No characterization of sensitivity (e.g., comparative cholinesterase inhibition in adults and pups) was conducted at doses in the approximate dose range at which this chemical is regulated for dietary exposure (e.g., NOAELSs of 4 mg/kg/day and 50 mg/kg for chronic and acute dietary risk assessments, respectively); no LD50 values were lower than 177 mg/kg. In the course of FIFRA SAP review of the OPP 10X policy proposals (March, 1998, July, 1998 and May, 1999), cautions have been raised regarding the inappropriateness of basing a determination of age-related susceptibility solely on acute lethality data. Such information, when available can serve as an indicator that there may be some concerns and that additional testing may be needed to further

characterize the effect. Additionally, it can be used in conjunction with other data in the weight-of-evidence discussions that are applied to the FQPA safety factor decision.

In Attachment 18, other studies are cited that appear to indicate that there is an age-related difference in sensitivity to Malathion. Two of these studies are comparisons of the median lethal dose: 1) a bovine acute lethality study conducted by American Cyanamid and 2) an acute interperitoneal study in rats (no source or author provided). A reference to a study by Brodeur and DuBoise (1963) states their conclusion that young animals were more sensitive than older animals, however, the supporting data are not described.

B. Retinal Rosettes

Dr. Dementi submitted Attachment 7 (January 15, 1998) to the HIARC after the *ad hoc* Neurotoxicology Subgroup had discussed this issue (November 13, 1997). It was noted in the attachment that "various published works indicate that the terms retinal rosette, retinal fold and retinal detachment **may** apply to the same or very similar anatomic or pathologic condition, all of which seem to involve a separation and folding of certain layers of retina". The cited investigators were Tansley (1933), Lai and Rana (1985), Rubin (1874) and Kuno et al. (1991). It is interesting to note the conjecture by these authors but there is nothing in the attachment which provides conclusive evidence that these terms can be used interchangeably.

The *ad hoc* Neurotoxicity Subgroup concluded that retinal rosettes in rats are most likely the result of abnormal proliferation and differentiation of developing retinal cells during neonatal life (i.e. during the first approximately 32 days after birth) and ordinarily are not likely to develop in mature animals as a result of treatment with xenobiotics.

C. Additional Questions Submitted to the External Peer Review Panel

Dr. Dobozy noted that the HIARC did not discuss three additional general questions submitted to the External Peer Review Panel. Evidently, they were submitted to the panel separately from the questions discussed in the HIARC report. Basically, the panel was asked to express their opinions with respect the appropriateness of the selected endpoints, the uncertainty factor used for the chronic RfD; the selection of the 2-year rat study for the chronic RfD and the completeness of the data base. The panel's responses to these questions are varied and often directed towards generic issues.

Although the panel's responses to these questions were not specifically addressed in the HIARC report. These issues are routinely considered during the HIARC review process and are discussed in as much detail as necessary to reach a scientifically sound decision.